

Claims

1. A bovine beta-casein gene targeting vector comprising
(1) a first region having a length of 5 to 12 kb which is
5 homologous to the promoter and its flanking nucleic acid
sequences of bovine beta-casein gene, and comprising exon 1,
intron 1, and exon 2 of bovine beta-casein gene; (2) a
region for cloning a nucleic acid coding for desired
proteins; (3) a region for coding a positive selection
10 marker; (4) a second region having a length of 2.8 to 3.5
kb which is homologous to the nucleic acid sequences of
bovine beta-casein gene, and comprising exon 5, 6, 7 and 8,
and intron 5, 6 and 7 of bovine beta-casein gene; wherein
the nucleic acid segment corresponding to the first region
15 is located upstream to the nucleic acid segment
corresponding to the second region in the 5'-3' arrangement
of beta-casein gene.
- 20 2. The vector according to claim 1, wherein the length of
the first region is 5.5 to 10kb.
3. The vector according to claim 1, wherein the length of
the second region is 3.0 to 3.2 kb.
- 25 4. The vector according to claim 1, wherein the positive
selection marker is selected from the group consisting of
neomycin (Neo), hygromycin (Hyg), histidinol dehydrogenase
gene (hisD) and guanine phosphoseryltransferase (Gpt).

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5. The vector according to claim 1, wherein the vector further comprises a region for a negative selection marker.

5 6. The vector according to claim 5, wherein the negative selection marker is Diphtheria toxin (DT) gene.

7. A vector according to claim 1 or 5 which is pBCKI I, pBCKI II, pBCKIDT I or pBCKIDT II, is presented in FIG. 1, FIG.
10 2, FIG. 16, or FIG. 3, respectively.

8. A bovine somatic cell which is beta-casein gene-targeted with the vector according to claim 1 or 5.

15 9. An embryo which is nuclear-transferred with the bovine somatic cell according to claim 8.

10. A method for producing a bovine beta-casein gene-targeted somatic cell which comprises the steps of (1)
20 introducing the bovine beta-casein gene-targeting vector according to claim 1 or 5 into an bovine somatic cell; (2) occurring homologous recombination events in the bovine somatic cell; and (3) selecting the bovine beta-casein gene-targeted somatic cell with a desired gene by
25 homologous recombination.

11. The method according to claim 10, wherein the vector in the step (1) is introduced into cells in form of linearized or deleted form lacking plasmid vector backbone.

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12. A method for generating transgenic cattle which
comprise the steps of (1) introducing the bovine beta-
casein gene-targeting vector according to claim 1 or 5 into
a bovine somatic cell; (2) occurring homologous
5 recombination events in the bovine somatic cell; (3)
selecting the bovine beta-casein gene-targeted somatic cell
with a desired gene by homologous recombination; (4)
introducing the gene-targeted cell into a nuclear-removed
bovine embryo to produce a nuclear-transferred embryo ; and
10 (5) implanting the embryo into a recipient.

13. A method obtaining a large scale of desired proteins
from milk of the transgenic cattle, in accordance with the
method of claim 12.

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